Pharmacotherapies for the treatment of glioblastoma - current evidence and perspectives

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Abstract: INTRODUCTION Glioblastoma, the most common malignant brain tumor, exhibits a poor prognosis with little therapeutic progress in the last decade. Novel treatment strategies beyond the established standard of care with temozolomide-based radiotherapy are urgently needed. AREAS COVERED We reviewed the literature on glioblastoma with a focus on phase III trials for pharmacotherapies and/or innovative concepts until December 2015. EXPERT OPINION In the last decade, phase III trials on novel compounds largely failed to introduce efficacious pharmacotherapies beyond temozolomide in glioblastoma. So far, inhibition of angiogenesis by compounds such as bevacizumab, cediranib, enzastaurin or cilengitide as well as alternative dosing schedules of temozolomide did not prolong survival, neither at primary diagnosis nor at recurrent disease. Promising strategies of pharmacotherapy currently under evaluation represent targeting epidermal growth factor receptor (EGFR) with biomarker-stratified patient populations and immunotherapeutic concepts including checkpoint inhibition and vaccination. The clinical role of the medical device delivering 'tumor-treating fields' in newly diagnosed glioblastoma which prolonged overall survival in a phase III study has remained controversial. After failure of several phase III trials with previously promising agents, improvement of concepts and novel compounds are urgently needed to expand the still limited therapeutic options for the treatment of glioblastoma.

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Pharmacotherapies for the treatment of glioblastoma – current evidence and perspectives

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Abstract

Introduction

Glioblastoma, the most common malignant brain tumor, exhibits a poor prognosis with little therapeutic progress in the last decade. Novel treatment strategies beyond the established standard of care with temozolomide-based radiotherapy are urgently needed.

Areas covered

We reviewed the literature on glioblastoma with a focus on phase III trials for pharmacotherapies and/or innovative concepts until December 2015.

Expert opinion

In the last decade, phase III trials on novel compounds largely failed to introduce efficacious pharmacotherapies beyond temozolomide in glioblastoma. So far, inhibition of angiogenesis by compounds such as bevacizumab, cediranib, enzastaurin or cilengitide as well as alternative dosing schedules of temozolomide did not prolong survival, neither at primary diagnosis nor at recurrent disease.

Promising strategies of pharmacotherapy currently under evaluation represent targeting epidermal growth factor receptor (EGFR) with biomarker-stratified patient populations and immunotherapeutic concepts including checkpoint inhibition and vaccination. The clinical role of the medical device delivering "tumor-treating fields" in newly diagnosed glioblastoma which prolonged overall survival in a phase III study has remained controversial. After failure of several phase III trials with previously promising agents, improvement of concepts and novel compounds are urgently needed to expand the still limited therapeutic options for the treatment of glioblastoma.

Article highlights

- For patients with newly diagnosed glioblastoma, the standard of care remains temozolomide-based radiochemotherapy.
- Phase III data on bevacizumab, cilengitide, and alternative dosing schedules for temozolomide did not show a survival benefit in newly diagnosed glioblastoma
- For elderly patients with a methylated MGMT promoter, temozolomide alone is probably superior to radiotherapy alone
- In recurrent disease, no widely accepted standard of care exists. Alkylating chemotherapy either as a rechallenge with temozolomide or nitrosoureas (e.g. CCNU), and bevacizumab are currently used. The combination of bevacizumab with CCNU is not superior for OS to single agent activity of CCNU.
- Phase III data emerging in the next years will define the role of novel immunotherapeutic concepts including checkpoint inhibition and vaccination

1. Introduction

Glioblastoma, the most common malignant primary brain tumor, has an incidence of 3.2 per 100,000 with predominance in males according to the Central *Brain Tumor Registry* of the United States (*CBTRUS*, 2008-2012) [1]. The prognosis for patients with glioblastoma has remained poor despite multimodal therapy. Median overall survival (OS) in a population-based study in the US after the introduction of the standard of care consisting of surgery plus temozolomide (TMZ)-based radiochemotherapy in 2005 was 9.7 months for the time frame of 2005-2008 [2]. In the Canton of Zurich, Switzerland, median OS in patients diagnosed between 2005 and 2009 was 11.1 months [3]. Poor prognostic factors include low performance status, high age, less than gross total resection, and among molecular markers an unmethylated promoter of the DNA repair gene *O^6-methylguanine-DNA methyltransferase* (*MGMT*) as well as wildtype *isocitrate dehydrogenase (IDH)-1/2* status [4].

During the course of the disease, despite intense initial treatment, tumor recurrence almost inevitably occurs. For recurrent disease, no widely accepted standard of care exists. Nitrosoureas, TMZ rechallenge or bevacizumab are among the currently used medical options for the treatment of recurrent glioblastoma [5-7].

Main challenges in developing efficacious pharmacotherapies for glioblastoma include high genetic heterogeneity within the tumor and between different patients, rapid development of drug resistance and poor distribution of most drugs within the brain [8, 9].

In this review, we summarize the literature on glioblastoma until December 2015 focussing on phase III trials for pharmacotherapies or innovative concepts or compounds currently used in the disease. Table 1 gives an overview on the data on completed phase III trials in newly diagnosed glioblastoma since 2005.

2. Alkylating chemotherapy

2.1. Temozolomide and the standard of care in newly diagnosed glioblastoma

TMZ, an imidazotetrazinone prodrug, is characterized by 100% bioavailability in plasma after oral intake and proven penetration into the cerebrospinal fluid with about 20% of the area under the curve (AUC) of that reached in plasma [10]. Its active metabolite, 5-(3-methyl triazen-1-yl)imidazole-4-carboxamide, mediates the cytotoxic effect by methylation of the O^6 position of guanine with additional alkylation at the N^7 position [10, 11].

The landmark trial in the treatment of glioblastoma represents the phase III trial of the European Organisation for Research and Treatment of Cancer (EORTC)/National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) establishing the current standard of care and leading to approval of TMZ in newly diagnosed disease [12]. The addition of TMZ during and after radiotherapy resulted in prolonged median OS of 14.6 months compared to radiotherapy alone (12.1 months). An updated analysis after a median follow-up of more than 5 years confirmed the efficacy of TMZ showing an OS fraction at 2 years of 27.2%, and 9.8% at 5 years in the TMZ arm, versus 10.9% at 2 years and 1.9% at 5 years with radiotherapy alone [13]. The administration schedule of the drug in this trial consisted of concomitant TMZ during radiotherapy (75 mg/m^2 daily) and up to six cycles of adjuvant TMZ (150-200 mg/m^2 for 5 days every 28 days). Toxicity of the drug was mainly hematologic (16% grade 3/4 events) [12]. The survival benefit in the TMZ group was mainly restricted to patients with a methylated *MGMT* promoter in the tumor with an OS of 21.7 months versus 15.3 months in patients without methylated *MGMT* promoter establishing the role of *MGMT* as a predictive biomarker in glioblastoma [14].

To improve the efficacy of TMZ and to overcome resistance to TMZ, alternative administration schedules, especially with dose intensification have been evaluated in clinical trials. One large randomized phase III trial compared the standard schedule of adjuvant TMZ (150-200 mg/m^2 for 5 days every 28 days) with a dose-dense schedule (TMZ 75-100 mg/m2 for 21 days every 28 days) in newly diagnosed glioblastoma. No difference in overall survival

but increased hematologic toxicity was observed. *MGMT* promoter methylation did not predict benefit from intensified TMZ compared to the standard schedule in this trial but was associated with prolonged OS independent of treatment [15]. For years, it has remained controversial whether extending TMZ beyond the six cycles of the standard schedule would improve outcome. A pooled analysis of 4 randomized clinical trials (EORTC/NCIC 26981-CE.3; EORTC26071-CENTRIC; EMD-CORE; RTOG 0525-Intergroup) did not show a benefit for OS if TMZ was extended beyond 6 cycles, including patients with tumors with *MGMT* promoter methylation [16]

More recently, it was demonstrated by two randomized trials that in elderly patients, e.g. older than 60-65 years, TMZ monotherapy in patients with glioblastoma with a methylated *MGMT* promoter was superior to radiotherapy alone having been the standard of care in this patient population for decades [17-19]. Based on these data, current guidelines were changed towards a biomarker-driven decision-making in elderly patients with newly diagnosed glioblastoma: For patients with glioblastomas with an unmethylated *MGMT* promoter or unknown methylation status, hypofractionated radiotherapy alone remains the standard of care while in patients with tumors with a methylated *MGMT* promoter, TMZ without or with radiotherapy should be preferred [7]. Whether combined chemoradiotherapy in the elderly patient population is superior to radiotherapy alone is currently under evaluation in a randomized phase III trial of the NCIC-CTG and EORTC (NCT00482677) [20]. Regarding the radiation schedule, a phase III trial with 98 elderly and/or frail patients with low Karnofsky performance status (50-70%) demonstrated that a one-week course with 25 Gy in five daily fractions was not inferior to standard three-week radiotherapy (total of 40 Gy) [21]. Yet, no effort of correlation with molecular markers was made in this study, and quality of life data were inconclusive.

In recurrent glioblastoma, TMZ had been already used prior to its approval in newly diagnosed disease based on the data of 2 phase II trials, one single arm study and one

randomized trial showing superiority to procarbazine [22, 23]. After establishment of the standard of care with TMZ in the newly diagnosed setting, different regimens of TMZ reexposure, especially dose-intensified schedules were evaluated for recurrent disease [24]. Most trials were small uncontrolled single-arm studies with heterogeneous patient populations with a median OS ranging between 5.1 and 11.7 months in TMZ-pretreated patients [25-27]. The DIRECTOR trial did not find any difference in OS comparing two dose-intensified schedules in patients with recurrent glioblastoma (TMZ 80 mg/m2 for 21 days out of 28 days versus TMZ 120 mg/m^2 for 7 days out of 14 days) [28]. Yet, DIRECTOR showed that dose-intensified TMZ provides relevant tumor control only in patients with tumors with MGMT promoter methylation. In the absence of convincing data, the overall clinical benefit from dose-intensified TMZ regimens compared to the standard schedule remains doubtful.

2.2 Nitrosoureas

Nitrosoureas are a group of DNA alkylating agents characterized by their high lipid solubility and thereby ability to cross the blood-brain-barrier which led to their use in brain tumors for decades. Carmustine (BCNU), nimustine (ACNU) and fotemustine are administered intravenously while lomustine (CCNU) is given orally. Toxicity, especially bone marrow suppression and hepatic toxicity, is more prominent than with TMZ.

Before the approval of TMZ in newly diagnosed glioblastoma, nitrosoureas were frequently combined with radiotherapy in the first-line setting [29]. Following the introduction of TMZ in the first-line setting, nitrosoureas have been mainly used at recurrence.

In the last decade, several randomized clinical trials in recurrent glioblastoma chose nitrosoureas, especially CCNU as active comparator for the experimental drug.

Median OS ranged between 7.1 and 9.8 months [25, 30-32]. None of the novel pharmacotherapies proved superiority to nitrosoureas so far, indirectly confirming their

activity for recurrent disease. Still, relevant toxicity, especially bone marrow suppression, limits the use of nitrosureas in glioblastoma patients.

Gliadel® wafers are a specific local application mode of BCNU shown to prolong survival in recurrent malignant glioma [33] and in newly diagnosed malignant glioma [34]. No survival benefit was seen in the latter trial when the analysis was restricted to glioblastoma only. Although approved in many countries in the world, the use of Gliadel® wafers has probably constantly declined over the last years.

3. Antiangiogenic therapy

Since glioblastomas are highly vascularized tumors, pharmacotherapies inhibiting angiogenesis emerged as a promising therapeutic strategy in the last decade. Main ideas of that concept included to starve the tumor by disrupting its vessels, to induce vessel "normalization" with improved delivery of chemotherapy and to fight a non-neoplastic target with limited intrinsic development of resistance.

However, all clinical trials in glioblastoma conducted so far failed to prove efficacy of anti-angiogenic compounds with regard to prolonged OS.

3.1 Bevacizumab alone and combination therapy

The pharmacological compound inhibiting angiogenesis characterized best in glioblastoma and also in other tumors represents bevacizumab, a humanized monoclonal antibody against the vascular endothelial growth factor (VEGF). Data on pharmacokinetic and bioavailability of the drug especially with regard to target inhibition are limited. Serum levels as well as the half-life of the drug in tumor patients are highly variable [35]. Based on the molecular structure, no penetration across an intact blood-brain-barrier is expected. However, in glioblastoma, a disrupted blood-brain-barrier may lead to an intratumoral delivery of the drug.

In clinical trials, dosing varied between 5 and 15 mg/kg every 2 or 3 weeks with the most common dose of 10 mg/kg every 2 weeks.

Typical adverse events associated with bevacizumab include arterial hypertension, thromboembolic events, cerebral hemorrhage, impaired wound healing and intestinal perforation [36, 37].

In newly diagnosed glioblastoma, two large phase III trials (AVAGlio, RTOG 0825) evaluating TMZ-based radiochemotherapy with or without bevacizumab failed to prove a benefit in OS [36, 37]. In both studies, a composite primary endpoint of OS and progression-free survival (PFS) was chosen. In contrast to OS, a prolongation of PFS was observed in both trials, by 4.4 months (AVAGlio), or by 3.4 months (RTOG 0825), although not reaching formal significance in the RTOG trial. The discrepancy between prolongation of PFS but not OS remains poorly understood. In part, crossover effects (31% AVAGlio, 48% RTOG 0825) may have contributed to this disconnect. In addition, determination of progression by radiological response criteria is still a controversial topic in neurooncology and may potentially be misleading in antiangiogenic therapies. The respective trials used different response criteria (RTOG 0825, Macdonald criteria/ AVAGlio, adapted RANO-criteria including independent imaging review). Subgroup analyses were performed in order to identify those patients likely to benefit from bevacizumab. In the RTOG 0825 trial, neither the MGMT status nor a prespecified 9-gene signature identified differences between the study groups [36]. In the biomarker population of the AVAGlio trial, IDH1 wild-type tumors were analysed for subtypes according gene expression profiles suggesting a benefit of bevacizumab in the proneural subtype in contrast to the mesenchymal or proliferative subtypes [38]. Still, this was an exploratory endpoint needing confirmation in a clinical trial with corresponding pre-specified patient stratification.

Several combination approaches have been explored in order to improve the efficacy of antiangiogenic drugs, especially of bevacizumab. In newly diagnosed glioblastoma, the

Glarius trial evaluated in a multicenter phase II design the combination of bevacizumab and irinotecan against standard TMZ-based radiochemotherapy in patients with glioblastoma harboring an unmethylated *MGMT* promoter. PFS (5.9 versus 9.7 months) but not OS (16.6 versus 17.3 months) was significantly prolonged [39].

In recurrent glioblastoma, bevacizumab was approved in the US and many other countries but not in the European Union based on the results of 2 phase II trials achieving radiographic response rates around 30% and PFS-6 rates of 42.6 and 29%, respectively [40, 41]. However, the effect of bevacizumab on OS remained uncertain since these trials lacked a study arm without the experimental drug. The first clinical trial in recurrent glioblastoma evaluating bevacizumab with a bevacizumab-free control arm represents the Belob trial [42]. Since this trial was conducted in the Netherlands where bevacizumab is not approved, cross-over effects were virtually absent. The trial was designed as a three-arm study comparing bevacizumab versus CCNU versus the combination of both drugs. The outcome with a median OS of 12 months of the combination arm versus 8 months of each of the monotherapy arms suggested a comparable activity of single agent bevacizumab and CCNU but importantly a benefit of the combination treatment. Based on these results, the design of the EORTC-26101 trial was adapted. The trial was initially planned as a phase II study with 4 arms randomizing for bevacizumab followed by CCNU, CCNU followed by either bevacizumab or best-investigator's choice or the combination of both followed by best-investigator's choice after progression on the first regimen. In the adapted design, the combination of bevacizumab and CCNU was tested in phase III randomized fashion versus CCNU as a single agent in patients with first recurrence of glioblastoma. The outcome data were presented at the 2015 Society for Neuro-Oncology (SNO) Meeting. The primary endpoint, OS, was not significantly different between CCNU monotherapy (median OS 8.6 months, n=149 patients) and the combination with bevacizumab (9.1 months, n=288 patients, hazard ratio (HR) 0.95). PFS was longer in the combination arm (4.2 months, HR 0.49) compared to CCNU alone (1.5

months). Crossover to bevacizumab in the monotherapy arm occurred in 35.5% while 19% of the patients in the combination arm continued bevacizumab after tumor progression [32]. Subgroup analyses, especially regarding *MGMT* promoter methylation and gene expression profiling, will be provided with the final report of the study.

In the past, many clinical trials evaluated other drug combinations to improve the efficacy of bevacizumab as a single agent in recurrent glioblastoma. So far, both cytotoxic chemotherapy, alternative antiangiogenic agents and targeted therapy failed to show substantial effects beyond single agent activity [25]. One compound merits to be mentioned: VB-111, a non-replicating adenovirus vector with modified murine promoter expressing a proapoptotic human Fas-chimera in order to target endothelial cells with potential antiangiogenic effects was safe in phase I/II in patients with recurrent glioblastoma [43, 44]. The initial design of the phase II trial comprised monotherapy with VB-111 ($3x10^{12}$ or $1x10^{13}$ viral particles every 2 months) until progression followed by bevacizumab. The protocol was amended to continue VB-111 with add-on of bevacizumab upon tumor progression. Overall survival was 15 months for the group with combination therapy at progression (n=24) versus 8 months for bevacizumab alone upon progression (n=22)[45]. Based on these results, a phase III trial comparing VB-111 combined with bevacizumab vs. bevacizumab is currently conducted in patients with recurrent glioblastoma (NCT02511405).

A common clinical practice in the use of bevacizumab is to continue the drug beyond progression on the drug to avoid potential rebound effects based on little retrospective data with high probability of selection bias. The phase II CABARET trial evaluated the outcome of patients randomized to continue or stop bevacizumab upon progression on either bevacizumab alone or combined with carboplatin in patients with recurrent glioblastoma. No differences in PFS or OS were reported [46].

3.2 Other anti-angiogenic agents evaluated in phase III trials: Cediranib, cilengitide, enzastaurin

Beyond bevacizumab several other drugs targeting factors involved in angiogenesis have been evaluated for the treatment of newly diagnosed or recurrent glioblastoma.

Cediranib, a tyrosine kinase inhibitor of VEGF receptor-1,-2,-3, PDGFR and c-kit, showed similar results regarding PFS rates in phase II as bevacizumab [47]. However, neither as a single agent nor in combination with CCNU, it was superior to CCNU monotherapy in a phase III trial in recurrent glioblastoma [31].

For cilengitide, targeting the integrins αvβ3 and αvβ5 with a putative antiangiogenic effect, two phase II trials showed encouraging results with a median OS of 16.1 and 19.7 months in newly diagnosed glioblastoma when combined with standard radiochemotherapy and 9.9 months at recurrence [48-50]. Subgroup analyses suggested a benefit of cilengitide specifically in patients with tumors harboring a methylated *MGMT* promoter [50]. Based on these results, a phase III trial (CENTRIC) in patients with newly diagnosed glioblastoma with methylated *MGMT* promoter evaluating cilengitide with and without standard radiochemotherapy was conducted. The results were disappointing, showing no difference in OS or PFS [51]. A randomized phase II study with 265 patients compared two different dosing regimens of cilengitide in combination with standard radiochemotherapy with the standard of care alone in patients with newly diagnosed glioblastoma and an unmethylated *MGMT* gene promoter. Since there was no dose-dependent effect on outcome with non-significant effects of the high dose of cilengitide on PFS and OS, no clear signal for efficacy in this patient population was found either [52].

Enzastaurin represents another compound potentially inhibiting angiogenic pathways in glioblastoma. It is an ATP-competitive inhibitor of protein kinase C-beta involved in downstream signaling of VEGF and other pathways. In newly diagnosed glioblastoma, a

single arm study for patients without methylation of the MGMT promoter missed its primary endpoint with a PFS-6 rate of 53.6% [53].

In recurrent glioblastoma, phase II data showed radiographic response rates of 25% but limited 6-months PFS of 7% [54]. The subsequent phase III trial for recurrent disease was stopped after poor results of an interim analysis without significant differences in PFS and OS compared with CCNU [30].

In conclusion, after failure in phase III, there is no evidence for clinical efficacy in glioblastoma of cediranib, cilengitide or enzastaurin.

4. Other targeted therapies

Agents targeting pathways involved in the pathogenesis of glioblastoma, in part with promising effects in other types of cancer, have been extensively evaluated in glioblastoma. A detailed discussion of these strategies, recently provided [9], would be beyond the scope of this review. Most concepts were not developed beyond phase II because of disappointing results.

An inhibitor of MGMT, O^6benzylguanine (O^6BG), was tested in a phase III design in combination with BCNU plus radiotherapy versus BCNU plus radiotherapy alone with the hypothesis to sensitize glioma cells to alkylating chemotherapy. This trial, conducted in the pre-TMZ era, was negative both with lack of OS benefit and additional toxicity in the experimental arm [55].

For patients with IDH1 mutated tumors, small molecule inhibitors of IDH1 are in clinical development. Since this mutation is an early event in tumorigenesis and absent in healthy tissue, it represents an ideal pharmacological target [56]. To date, small molecule inhibitors such as AG-881 and AG-120 are in phase I development (NCT02481154, NCT02073994). First results of the phase I study of AG-120 in patients with IDH-mutated solid tumors including glioma (NCT02073994) were presented at the AACR-NCI-EORTC International

Conference on Molecular Targets and Cancer Therapeutics 2015. The drug was well tolerated and 10 of the 20 patients with gliomas showed stable disease including 4 out of 11 patients with high-grade glioma [57].

Epidermal growth factor receptor (EGFR) plays a major role in the pathogenesis of glioblastoma and has been tried as a target for therapeutic purposes for years despite several negative trials, including tyrosine kinase inhibitors such as erlotinib or gefitinib [58, 59] or monoclonal antibodies such as nimotuzumab [60]. However, these trials were not conducted in biomarker-selected populations overexpressing the target. Recently, in a phase II trial in recurrent glioblastoma evaluating the ErbB family blocker afatinib, a small efficacy signal in EGFRvIII-positive tumors versus -negative tumors was seen (median PFS 3.4 versus 1.0 months) [61]. Currently tested in phase IIb/III is ABT-414, an antibody-drug conjugate targeting EGFR in its active conformation. The toxicity profile of the drug as assessed in phase I trial includes ocular adverse events such as corneal deposits and keratitis. In this trial, 5 of 18 patients in the cohort combined with TMZ and 2 of 28 patients in the monotherapy cohort had objective response. Importantly, all patients with documented radiographic response had EGFR amplification [62]. Therefore, in the subsequent clinical trials, only patients with amplified EGFR were included. In patients with newly diagnosed glioblastoma, the currently recruiting phase IIb/III trial evaluates the combination of ABT-414 with standard radiochemotherapy compared to the standard of care alone (NCT02573324). In recurrent gliobastoma, patients are randomized to ABT-414 alone or combined with TMZ and to CCNU or TMZ (NCT02343406, EORTC 1410).

5. Immunotherapeutic concepts

Currently the most promising field in oncology in general as well in glioblastoma represents immunotherapy including vaccination approaches and inhibition of immunosuppressive molecules [63].

Vaccination approaches aim at mounting a tumor-specific immune response, e.g. via peptides derived from tumor-specific antigens or cell-based approaches. The most popular and best characterized peptide-based approach represents the vaccination against the variant III of EGFR (EGFRvIII) which is present in about 25% of glioblastomas but absent in normal tissue. Rindopepimut is a vaccine comprising of an EGFRvIII-derived peptide conjugated to keyhole limpet hemocyanin (KLH) serving as a carrier and is administered intradermally with granulocyte macrophage colony-stimulating factor (GM-CSF) as adjuvant. A phase II trial in patients with newly diagnosed glioblastoma (ACT III), initially planned in a randomized but open-label fashion, evaluating the addition of Rindopepimut to standard radiochemotherapy versus radiochemotherapy alone, was changed to a one-arm design due to high drop-out rates in the control arm. Gross total resection, minimal residual disease ≤ 1 cm^2 and absence of tumor progression after completion of radiation therapy were required for study participation. The safety profile was favorable except for local skin reactions at the injection site. PFS at 5.5 months after study entry, the primary endpoint of the trial, was 66%. Median overall survival of 21.8 months from study entry was encouraging, although the highly selected patient population has to be taken into account. Importantly, anti-EGFRvIII antibody titers were increased at least 4-fold in 85% of the patients and EGFRvIII was eliminated in 4/6 (67%) tumor samples at recurrence after vaccination [64]. These data confirm results from 2 smaller phase II trials assessing rindopepimut in patients with newly diagnosed glioblastoma [65, 66]. The recently completed phase III trial (ACT IV, NCT01480479) was conducted in a double-blind placebo-controlled fashion including a placebo vaccine containing KLH. Recently, a press release of the manufacturer reported that the trial was discontinued in March 2016 since the study was unlikely to meet its primary endpoint (OS). Median OS of the experimental arm (20.4 months) was similar as in prior phase II trials, however, the control arm reached comparable results (21.1 months, HR 0.99)[67].

Although the vaccination is thought to work best when used early in the disease with minimal residual tumor, rindopepimut has also been evaluated in recurrent glioblastoma. The Re-ACT trial randomized patients to bevacizumab plus control vaccine versus bevacizumab combined with rindopepimut. Patients in the rindopepimut arm had higher overall response rates (30% vs. 18%), PFS-6 (28% vs. 16%) and prolonged median OS (11.3 vs. 9.3 months, HR=0.57). Potent anti-EGFRvIII immune titer generation was associated with prolonged OS [68], which might also simply reflect immunological fitness as a prognostic factor.

Another promising neoantigen suitable for peptide-based vaccination represents mutated IDH1 (IDHR132H), a mutation occurring early in tumorigenesis and virtually absent in normal tissue. Preclinical models support this concept [69]. The currently recruiting phase I trial NOA-16 evaluates safety and immune response to the IDH1 peptide vaccine in patients with IDH1-mutated WHO grade III and IV gliomas (NCT02454634).

Another approach to induce anti-tumor immunity which will be assessed in a phase III trial is the compound ICT-107, a vaccine of patient-derived dendritic cells incubated with peptides derived from 6 tumor-associated antigens (melanoma-associated antigen 1 (MAGE-1), human epidermal growth factor receptor 2 (HER2), absent in melanoma 2 (AIM-2), tyrosinase-related protein-2 (TRP-2), glycoprotein100 (gp100), and interleukin-13 receptor subunit alpha-2 (IL-13Rα2). Phase I data showed good tolerability, immune responses were demonstrated in one third of the patients [70]. The subsequent phase II trial randomized ICT-107 in a 2:1 manner in addition to standard radiochemotherapy in patients with newly diagnosed glioblastoma. This study showed a significantly improved PFS in the experimental arm [71]. In the subgroup of HLA-A2-positive patients immunologic response was associated with a median OS of 23.1 months for responders and 13.7 for non-responders [72]. Based on these encouraging results, a multi-center randomized, double-blind phase III trial in HLA-A2-positive patients with newly diagnosed glioblastoma has been initiated (NCT02546102).

Another vaccine based on dendritic cells, DCVax®, is currently evaluated in a phase III trial (NCT00045968). The therapeutic priniciple, injecting patient-derived dendritic cells pulsed with autologous tumor lysate was safe in a phase I trial [73].

Beyond vaccination, the currently most promising strategy in cancer immunotherapy represents immune checkpoint inhibition. These compounds target inhibitory immune cell receptors and their ligands such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), the receptor programmed cell death-1 (PD-1) or its ligand PD-L1 aiming at overcoming tumor-induced immune tolerance. After the success of the first compound in this field, ipilimumab, an antibody to CTLA-4, in metastatic melanoma [74], a plethora of agents has entered the clinic in cancer therapy and clinical trials. A phase III trial evaluating nivolumab, an antibody targeting PD-1, versus bevacizumab in patients with recurrent glioblastoma has recently completed accrual (Checkmate 143, NCT02017717). Within a small safety cohort of this trial, the OS rate at 6 months was 70% for patients receiving nivolumab alone. Monotherapy of nivolumab was well tolerated while the combination with ipilimumab was discontinued at phase I because of limiting toxicity including colitis, cholecystitis, diabetic ketoacidosis, and confusion [75]. Nivolumab will also be tested in patients with newly diagnosed glioblastoma. A phase III trial for patients with glioblastoma and unmethylated MGMT promoter will compare standard TMZ-based radiochemotherapy with radiotherapy and nivolumab (Checkmate 498, NCT02617589). A companion trial for patients with MGMT promoter-methylated glioblastoma is planned. Similar compounds are under development, e.g. pembrolizumab (NCT02337491) or MEDI4736 (NCT02336165) currently evaluated in phase II.

6. Gene therapy

Virus-delivered gene therapies may in part mediate their effects via immunological mechanisms, too. A phase III trial evaluated a locally applied adenovirus-mediated gene

therapy with a prodrug converting enzyme (herpes-simplex-virus thymidine kinase; sitimagene ceradenovec) followed by intravenous ganciclovir in patients with newly diagnosed resectable glioblastoma in addition to standard radiochemotherapy compared to the standard of care alone. The co-primary endpoint of the trial, median time to death or re-intervention, was prolonged in the experimental group (308 days) relative to the control group (268 days), in contrast to OS which was not different in both arms [76].

7. Tumor treating fields

Beyond pharmacotherapy, a novel modality of anti-tumor therapy was tested in glioblastoma: tumor-treating fields (TTFields/NovoTTF) represents a portable device to be carried by the patient for at least 18-20 h per day. The device delivers alternating electric fields of low-intensity and intermediate frequency supposed to have anti-mitotic effects. A randomized phase III trial in newly diagnosed glioblastoma demonstrated prolonged PFS (7.1 versus 4.0 months) and OS (19.6 versus 16.6 months) for the addition of TTFields (>18h/day) to maintenance TMZ [77]. Accrual was stopped early for success after a pre-specified interim analysis on 315 patients. The design did not include blinding or placebo-control of the device and excluded patients with poor outcome since randomization became effective only after completion of radiotherapy and required demonstration of stable disease at that timepoint.
A randomized phase III trial in recurrent glioblastoma had previously evaluated TTFields (>20h/day) versus best physician's choice of chemotherapy. OS and PFS were not significantly different [78]. There were no major limitations regarding toxicity in both trials except for mild to moderate skin reactions. The FDA has approved the device both for newly diagnosed and recurrent glioblastoma. Since patient acceptance of this treatment is limited at present, the future place of TTFields in the treatment of glioblastoma remains to be defined.

7. Conclusion

In the last ten years, despite several phase III trials with previously promising compounds and concepts, little progress has been made in the treatment of patients with glioblastoma. So far, no concept added to standard TMZ-based radiochemotherapy resulted in an additional benefit, except for the prolongation of OS in one trial by the application of TTFields. Alkylating chemotherapy with TMZ using the standard schedule remains the standard of care in newly diagnosed glioblastoma. Yet, clinical trials in the subgroup of elderly patients changed the previous standard of care, that is, radiotherapy alone: in case of a methylated *MGMT* promoter, TMZ with or without radiotherapy is probably superior to radiotherapy alone. Phase III trials with antiangiogenic agents, such as bevacizumab, cilengitide, cediranib or enzastaurin were negative despite promising data in phase II studies.

At recurrence, TMZ rechallenge, nitrosoureas such as CCNU, or bevacizumab where available represent widely accepted therapeutic options. Of note, no compound so far showed superiority to CCNU in a phase III trial in recurrent glioblastoma. Targeted agents often already failed to give an efficacy signal at phase II stage. Various immunotherapeutic concepts recently demonstrated encouraging results in phase I/II trials, efficacy results derived from randomized phase III trials will be available in the next 1-2 years.

8. Expert opinion

Improvement of the still poor prognosis of patients with glioblastoma remains a major challenge. Disease heterogeneity and rapid development of resistance limit the activity of pharmacological treatment. Despite advances in the understanding of the biology of the tumor, strategies in targeting key pathogenic mechanisms such as angiogenesis failed to prolong OS. Repeatedly, agents with promising data in phase II trials failed to confirm efficacy in randomized phase III trials. Table 2 shows current perspectives and summarizes clinical trials that will influence the field in the future. Alkylating chemotherapy with TMZ in

newly diagnosed glioblastoma and CCNU in recurrent disease will stay an important part of future clinical concepts and active comparators for the design of clinical trials. The clinical role of TTFields with positive results as an add-on to standard maintenance TMZ in newly diagnosed glioblastoma remains controversial. Key criticisms include the lack of blinding and placebo control in the phase III trial, which admittedly would have been a challenge for various reasons, and the early closure of the trial.

The perspectives of antiangiogenic compounds after the disappointing results of the clinical trials discussed above are uncertain. The future of bevacizumab, the most promising antiangiogenic agent, will depend on whether a subgroup of patients deriving benefit from the treatment and rational combination therapies can be defined. The identification of predictive biomarkers is the logical consequence of the increased molecular understanding of glioblastoma. Yet, beyond *MGMT* promoter methylation as a predictive biomarker for benefit from TMZ, no molecular marker or gene signature for benefit from tumor-specific treatment has been identified yet. Future efforts should aim at stratifying patients according pre-specified molecular subgroups with tailored therapeutic concepts. In line with this, the EGFR-targeted clinical trials, both the vaccination trial ACT-IV and the trial evaluating ABT-414, and clinical trials with IDH1 inhibitors or IDH1-targeted vaccinations are conducted in target-selected populations. However, the recently reported failure of the randomized vaccination trial ACT-IV evaluating rindopepimut, an EGFRvIII-targeted vaccine, is disappointing. Although phase II data had been promising, admittedly conducted in highly selected patient populations, the expectations in phase III were not met. Probably, using randomized trial designs already in phase II trials would help to identify concepts worth to be evaluated in phase III.

Despite the failure of ACT-IV, the currently most promising field in the pharmacological treatment of glioblastoma still represents immunotherapy. The success of checkpoint

inhibitors targeting CTLA-4, PD-1 or PD-L1 in other cancer entities encourages the currently conducted clinical trials with these agents in glioblastoma. Other promising immunotherapeutic concepts comprise the dendritic-cell based vaccine ICT-107 planned to be evaluated in phase III (NCT02546102) and an IDH1-targeted peptide vaccine in IDH1 mutated tumors (NCT02454634).

In conclusion, advances in the biological understanding of the tumor are urgently needed to be translated into clinically active compounds to improve the still limited therapeutic options in glioblastoma.

Table 1: Phase III trials in newly diagnosed glioblastoma published between 2005 and 2015

Clinical trial/ Reference	Patients	Investigative concept(s)	Mechanism of action	Treatment arms, no. of patients	Primary endpoint	mPFS (months)	mOS (months)	Status of the drug/conclusions
EORTC 26981/22981/ NCIC CE3 [12]	18 to 70 y	TMZ, standard schedule: during RT (75 mg/m^2/d, 6 cycles adjuvant (150-200 mg/m^2 for 5d, q28d)	Alkylation of DNA	TMZ/RT+TMZ 5/28 (n=286) *or* RT (n=287)	OS	6.9 (TMZ/RT+TMZ) 5.8 (RT)	14.6 (TMZ/RT+TMZ) 12.1 (RT)	TMZ approved for newly diagnosed glioblastoma, current standard of care *MGMT* promoter methylation predictive biomarker for benefit from TMZ
RTOG 0525 [15]	>18 y	TMZ, dose-intensification in adjuvant phase: 6-12 cycles adjuvant (75-100 mg/m^2 for 21d, q28d)	Alkylation of DNA	Standard TMZ 5/28 (n=411) *or* Dose-dense TMZ 21/28 (n=422)	OS	5.5 (Standard TMZ) 6.7 (Dose-dense TMZ)	16.6 (Standard TMZ) 14.9 Dose-dense TMZ)	TMZ approved for newly diagnosed glioblastoma No benefit of dose-dense schedule
SWOG S0001/ NCT00017147 [55]	>18 y	O^{6}BG 120 mg/m^2 +BCNU 40 mg/m^2 q6w	MGMT depleting agent (O^{6}BG), alkylation of DNA (BCNU)	RT+O^{6}BG 120 mg/m^2+BCNU 40 mg/m^2 (n=90) *or* RT+BCNU 200 mg/m^2+RT (n= 89)	OS	4 (each arm)	11 (O^{6}BG + BCNU) 10 (BCNU)	Trial stopped for futility at interim analysis No added benefit of O^{6}BG
NOA-08 [19]	>65 y	TMZ (100 mg/m^2 for 7d, q14d)	Alkylation of DNA	TMZ (n=195) *or* RT 30x1.8-2.0 Gy (n=178)	OS	3.3 (TMZ) 4.7 (RT)	8.6 (TMZ) 9.6 (RT)	TMZ or RT standard of care in patients >65y *MGMT* promoter methylation predictive biomarker for benefit of TMZ
Nordic [18]	>60 y	TMZ (150-200 mg/m^2 for 5d, q28d) RT 10x3.4 Gy	Alkylation of DNA (TMZ)	TMZ 5/28 (n=93) *or* RT 10x3.4 Gy (n=98) *or* RT 30x2 Gy (n=100)	OS	Not available	8.3 (TMZ 5/28) 7.5 (RT 10x3.4 Gy) 6.0 (RT 30x2 Gy)	TMZ or RT 10x3.4 Gy standard of care in patients >70y *MGMT* promoter methylation predictive biomarker for benefit of TMZ
AVAGlio/ [37]	>18 y	BEV (10 mg/kg q2w) in addition to TMZ/RT+TMZ	Antibody to VEGF	TMZ/RT+TMZ+BEV *or* TMZ/RT+TMZ+Placebo	OS+PFS	10.6 (BEV) *or* 6.2 (Placebo)	16.8 (BEV) *or* 16.7 (Placebo)	No approval of BEV for newly diagnosed glioblastoma Improved PFS and maintainance of quality of life (BEV)

RTOG 0825 [36]	>18 y	BEV (10 mg/kg q2w) in addition to TMZ/RT+TMZ	Antibody to VEGF	TMZ/RT+TMZ+BEV *or* TMZ/RT+TMZ+Placebo	OS+PFS	10.7 (BEV) *or* 7.3 (Placebo)	15.7 (BEV) *or* 16.1 (Placebo)	No approval of BEV for newly diagnosed glioblastoma Worse quality of life (BEV)
[21]	>65 y and/or KPS 50-70%	Short-course RT (5x5 Gy)	Radiation	RT 5x5 Gy (n=48) *or* RT 15x2.67 Gy (n=50)	OS	4.2 (both arms)	7.9 (5x5 Gy) 6.4 (5x2.67 Gy)	Short course RT noninferior to RT 5x2.67 Gy in elderly and/or patients with KPS 50-70%
CENTRIC/ [51]	>18 y, methylated *MGMT* promoter	CIL 2000 mg iv. twice/wk in addition to TMZ/RT+TMZ	Inhibitor of αvβ3/αvβ5 integrins	TMZ/RT+TMZ (n=273) *or* TMZ/RT+TMZ + CIL (n=272)	OS	10.7 (TMZ/RT+TMZ) 13.1 (CIL+ TMZ/RT+TMZ)	26.3 (TMZ/RT+TMZ) 26.3 (CIL+ TMZ/RT+TMZ)	No added benefit of CIL Drug development stopped for glioblastoma
OSAG 101-BSA-05 [60]	18 to 70 y	Nimotuzumab 400mg/wk for 12 wks, then twice/wk in addition to TMZ/RT+TMZ	Antibody to epidermal growth factor receptor	TMZ/RT+TMZ (n=74) *or* TMZ/RT+TMZ + nimotuzumab (n=75)	12-months PFS and PFS	5.6 (TMZ/RT+TMZ) 4.0 (Nimotuzumab+ TMZ/RT+TMZ)	19.5 (TMZ/RT+TMZ) 16.7 (Nimotuzumab+ TMZ/RT+TMZ)	No added benefit of nimotuzumab
ASPECT [76]	18 to 70 y	Intraoperative injection of sitimagene ceradenovec ($1x10^{12}$ viral particles) followed by ganciclovir 5 mg/kg iv. 2x/d in addition to TMZ/RT+TMZ	Adenovirus-mediated gene therapy	Experimental arm (n=124) *or* TMZ/RT+TMZ (126)	Time to death or re-intervention	Not available	16.2 (Experimental) 14.8 (TMZ/RT+TMZ)	No effect on overall survival of sitimagene ceradenovec
EF-14 [77]	>18 y	NovoTTF-100A after completion of TMZ/RT in addition to adjuvant TMZ	Alternating electric fields	TMZ/RT+TMZ +NovoTTF *or* TMZ/RT+TMZ	PFS	7.1 (TMZ/RT+TMZ +NovoTTF) 4.2 (TMZ/RT+TMZ)	19.4 (TMZ/RT+TMZ+ NovoTTF) 16.6 (TMZ/RT+TMZ)	Trial closed to accrual after interim analysis

Abbreviations: BEV, bevacizumab, CIL, cilengitide; d, days; mPFS, median progression-free survival; mOS, median overall survival; q, every; RT, radiotherapy; TMZ, temozolomide; VEGF, vascular endothelial growth factor; wk, week; y, years

Table 2: Current perspectives in the treatment of glioblastoma

Immunotherapy
• EGFRvIII-targeted vaccination (ACT-IV phase III, NCT01480479): discontinued, final results pending • Checkpoint inhibition: clinical trials ongoing or results pending: e.g. Nivolumab (phase III, NCT02017717), Pembrolizumab (Phase II, NCT02337491), MEDI4736 (Phase II, NCT02336165) • ICT-107 (dendritic cell-based vaccine): phase III (NCT02546102) • DCVax (Autologous dendritic cells pulsed with tumor lysate antigen): phase III (NCT00045968) • IDH-1 peptide vaccine in patients with IDH-1 mutated tumors (Phase I, NCT02454634, ongoing)
Alkylating chemotherapy
• No pharmacotherapy so far showed superiority to either TMZ in newly diagnosed glioblastoma or nitrosoureas in recurrent disease
Biomarker-driven decision making
• *MGMT* promotor methylation predictive for benefit from TMZ and used for decision making in elderly patients
Targeting amplified EGFR
• Phase IIb/III trial on ABT-414 in newly diagnosed glioblastoma (NCT02573324): recruiting • Phase II trial on ABT-414 in recurrent glioblastoma (NCT02343406): recruiting
Inhibition of angiogenesis
• No effect of BEV on OS demonstrated in phase III (AVAGlio, RTOG-0825, EORTC-26101) • Identify subgroups of patients deriving benefit of BEV • Potentially efficacious combinations: ▪ Re-ACT phase II: Rindopepimut+BEV ▪ NCT02511405: VB-111+BEV (Recruiting)
Alternative approaches
• EF-14 phase III trial (Novo-TTF)

Abbreviations: BEV, bevacizumab; TMZ, temozolomide

Abbreviations

AIM-2, absent in melanoma 2

EGFR, epidermal growth factor receptor

Gp100, glycoprotein100

HR, hazard ratio

HER2, human epidermal growth factor receptor 2

IDH, Isocitrate dehydrogenase

IL-13Rα2, interleukin-13 receptor subunit alpha-2

MAGE-1, melanoma-associated antigen 1

MGMT, O^6-methylguanine-DNA methyltransferase

PFS, progression-free survival

OS, overall survival

TMZ, temozolomide

TRP-2, tyrosinase-related protein-2

Declaration of interest

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